CLAIMS

1. A method of determining a nucleotide base in a nucleic acid sample comprising the steps of:

(i) incubating the nucleic acid sample with a primer, DNA polymerase deoxynucleotide triphosphate, or the corresponding deoxynucleotide triphosphate analogue or dideoxynucleotide triphosphate (representing a single base?)

(ii) measuring the pyrophosphate released in step (i)

(iii) identifying the nature of the base added by measuring which nucleotide caused the release of PPi in step (ii)

characterised in that steps (i) to (iii) are performed in a microfluidic device.

2. A method for identifying the sequence of a portion of sample DNA, which method comprises:

(i) forming immobilised double stranded DNA on one or more reaction areas in a microchannel structure of a microfluidic device:

(ii) adding a known deoxynucleotide (or the corresponding deoxynucleotide analogue or dideoxynucleotide) and a DNA polymerase to each of said one or more reaction areas so that extension of primer only occurs if there is a complementarity of the added deoxynucleotide or dideoxynucleotide with the strand of sample DNA that is part of the immobilised double stranded DNA;

(iii) detecting whether or not the deoxynucleotide or dideoxynucleotide added in step (ii) has been added to the primer DNA in said one or more reaction areas,

(iv) repeating steps (ii) and (iii) as required with a different deoxynucleotide (or the corresponding deoxynucleotide analogue or dideoxynucleotide).

3. A method of determining a nucleotide base in a nucleic acid sample

according to claim 1 or 2 comprising the steps of:

(i) attaching 0.1 – 200 pmol of a primer or single stranded DNA sample to each of between one and 100,000 pre-determined areas on the surface of a microfluidic device;

(ii) hybridising small amounts, e.g. 0.1 – 200 pmol, of single stranded sample DNA or primer respectively to each of the predetermined areas;

Sub A1

(iii) adding a known deoxynucleotide, deoxynucleotide analogue or dideoxynucleotide and a DNA polymerase so that extension of the primer only occurs, with consequent release of pyrophosphate (PPi), if there is a complementarity with the sample DNA;

(iv) measuring the release of PPi and from which predetermined area on the device it is released;

(v) repeating steps (iii) and (iv) as required to construct a DNA sequence for the elongated primers, and hence for portions of the sample DNA.

4. A method for identifying the sequence of a portion of sample DNA, which method comprises:

(i) adding sample DNA to a predetermined area on a microfluidic device

(ii) moving the sample to a reaction chamber on the microfluidic device

(iii) attaching the sample DNA to a surface of the reaction chamber, alternatively hybridising the sample DNA in a single stranded form to a primer attached to the reaction chamber (then to (v))

(iv) if the sample DNA has not been attached to a primer attached to the reaction chamber, hybridising a primer to the DNA in a single stranded form

(v) extending the primer in the presence of a DNA polymerase with a known deoxynucleotide (dNTP), deoxynucleotide analogue or dideoxynucleotide (ddNTP) such extension being indicated by

detection of pyrophosphate (PPi) released from the extension reaction

(vi) repeating step (v) as required to establish the sequence of the extended primer.

5. A method according to any one of claims 1, 3 or 4 wherein the pyrophosphate release is detected by light emitted from a luciferin luciferase reaction.

6. A method according to claim 2 wherein the detection step involves labelled terminator

7. A method Claim 1-6 wherein the detection of the deoxynucleotide/dideoxynucleotide incorporation is performed in real time.

8. A method according to any one of claims 1-7 wherein microfluidic devices is a disc wherein the fluids maybe moved by centripetal force.